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Abstract: Alternative reproductive tactics of males have been studied in many species, but few studies have focused on females. In many communally breeding mammals, females can be adult non-breeding helpers, leave the group and breed solitarily, or they could be a breeder in their natal group, representing three alternative reproductive tactics. The reasons for delayed breeding are not well understood, but in many sociable species both male and female helpers are reproductively suppressed. Male helpers of communally breeding striped mice (Rhabdomys pumilio) have increased corticosterone levels and delayed sexual maturation compared to their singly housed brothers. In the present study, we tested whether similar effects occur in female striped mouse helpers. In the field, young adult females typically do not breed in their natal group, indicating they might be reproductively supressed. Seventeen sister pairs from 17 family groups were studied. One sister of each pair was kept in the family group, while the other was housed singly at 3 weeks of age. Sisters did not differ in the age at which they reached puberty (on average at 6 weeks), their corticosterone levels, nor their progesterone levels. However, in neutral encounter tests, singly housed sisters showed more amicable behaviours when presented with unfamiliar striped mice of both sexes. Their high sociable motivation might explain why most females remain philopatric under natural conditions. We conclude that philopatric female striped mice in monogamous family groups arenot reproductively suppressed, but reproductive competition might occur in natural communal groups with multiple old breeding females, as observed under high population density.

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Absence of reproductive suppression in young adult female striped mice living in their natal family

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Alternative reproductive tactics of males have been studied in many species, but few studies have focused on females. In many communally breeding mammals, females can be adult non-breeding helpers, leave the group and breed solitarily, or they could be a breeder in their natal group, representing three alternative reproductive tactics. The reasons for delayed breeding are not well understood, but in many sociable species both male and female helpers are reproductively suppressed. Male helpers of communally breeding striped mice (*Rhabdomys pumilio*) have increased corticosterone levels and delayed sexual maturation compared to their singly housed brothers. In the present study, we tested whether similar effects occur in female striped mouse helpers. In the field, young adult females typically do not breed in their natal group, indicating they might be reproductively supressed. Seventeen sister pairs from 17 family groups were studied. One sister of each pair was kept in the family group, while the other was housed singly at 3 weeks of age. Sisters did not differ in the age at which they reached puberty (on average at 6 weeks), their corticosterone levels, nor their progesterone levels. However, in neutral encounter tests, singly housed sisters showed more amicable behaviours when presented with unfamiliar striped mice of both sexes. Their high sociable motivation might explain why most females remain philopatric under natural conditions. We conclude that philopatric female striped mice in monogamous family groups arenot reproductively suppressed, but reproductive competition might occur in natural communal groups with multiple old breeding females, as observed under high population density.

Keywords: Alternative reproductive tactics, corticosterone, helpers at the nest, reproductive suppression, *Rhadomys pumilio*, social flexibility, intra-specific variation in social organisation

In many animal species, individuals have the choice between alternative tactics, which are regarded to be the result of adaptive decision processes optimizing individual fitness (Dawkins, 1980; Gross, 1996). For individuals of social species, one such decision could be to choose between remaining philopatric as a helper or dispersing and start independent breeding (Koenig & Dickinsom, 2008). Such alternative reproductive tactics are more common in males than in females (Taborsky et al., 2008), perhaps because male reproductive success generally varies more between individuals than female reproductive success. Hoever, many sociable mammal species have non-breeding female helpers (Solomon & French, 1997), providing a good opportunity to study female tactics of helping versus dispersing and solitary breeding. In communally breeding species, more than one female breeds per group, and philopatric non-breeding female helpers might remain in these groups to start reproduction at a later stage (Hayes, 2000).

This absence of breeding in young adult females could be due to physiological reproductive suppression, defined as highly increased glucocorticoid levels induced by the presence of dominant breeders causing social stress (Creel, 2001; Reyer et al., 1986; Wingfield & Sapolsky, 2003). High corticosterone levels leadto suppression of progesterone secretion, thus inhibiting ovulation (Clarke et al., 2001; Saltzman et al, 2006). Reproductive suppression of subordinate female helpers occurs both in sevreal communally breeding species (Brant et al., 1998; Getz et al., 1983; Solomon et al., 2001).

Alternative reproductive tactics are characterized by behavioural differences between tactics, especially in reproductive and social behaviour. When some individuals disperse while others remain philopatric, they follow two alternative tactics that are also characterized by differences in the social environment: living in

68 the natal group, living solitarily, or emigrating into and living in a group of non-kin.
69 Thus, it is not well understood whether behavioural differences between tactics are
70 primarily due to motivational (internal) differences between individuals of the two
71 different tactics, or alternatively simply a consequence of the external differences in
72 social environment: individuals living in groups can show amicable behaviours
73 towards group mates, solitary individuals cannot. Therefore, to understand differences
74 in social behaviour between alternative tactics, it is important to study social
75 behaviour under standardized conditions, for example using a neutral presentation
76 arena.

77 In African striped mice (*Rhabdomys pumilio*), both sexes have three
78 alternative reproductive tactics (Schradin et al., 2012b): 1. philopatric helper, 2.
79 solitary breeder or 3. communal breeder. The group-living tactics 1 and 3 occur when
80 population density is very high, the solitary tactic when population density is very
81 low, and all tactics occur under intermediate population densities (Schradin et al.,
82 2010a). Under low population density, both males and females can leave their natal
83 group in the season of their birth when they are 4-6 weeks old to start independent
84 breeding (Schoepf & Schradin, 2012). Dispersing young adults were more aggressive
85 towards individuals of the same sex compared to philopatrics and more amicable
86 towards individuals of the opposite sex (Schoepf & Schradin, 2012b). In sum, free-
87 living young adult striped mice can either be group-living or solitary living, making
88 this species a prime model to test for reproductive suppression using an experimental
89 approach (Schradin et al., 2009, 2012a). Philopatric males are known to be
90 reproductively suppressed by the breeding male of the group (Schradin et al., 2009),
91 showing high corticosterone but low testosterone levels, small testes and low, if any,
92 sperm counts (Schradin et al., 2012a). However, we do not know whether philopatric

93 females are also reproductively suppressed, which could explain why many of them
94 do not breed as young philopatric adults.
95 In the present study, we set up 17 family groups to mimic the philopatric and
96 the solitary female tactic. From each family, two same litter sisters were used: one of
97 which remained philopatric with the family, while the other was housed singly after
98 reaching three weeks of age, the earliest age at which dispersal occurs (Schoepf &
99 Schradin, 2012a). This experimental design has been used previously and successfully
100 mimicked the philopatric and the solitary tactic in male striped mice, inducing
101 increased testosterone and decreased corticosterone levels in singly housed males
102 (Schradin et al., 2009), increased levels of stored arginine vasopressin in the brain
103 (Schradin et al., 2013), and enhanced testes development (Schradin et al., 2012a). The
104 present study had three aims. (I) We tested whether reproductive suppression occurs
105 in philopatric females by investigating whether philopatric females show delayed
106 onset of puberty (measured as age of first perforation of the vagina, indicating their
107 readiness to mate), higher corticosterone levels and lower progesterone levels. Such
108 reproductive suppression would explain why most young philopatric females do not
109 breed in nature. (II) We determined whether living alone or in the family group was
110 associated with differences in response to unfamiliar conspecifics. If the solitary tactic
111 is due to an internal motivation to avoid the company of conspecifics, we expected
112 solitary females to be more aggressive, less amicable and more explorative than
113 philopatric females. We further predicted that solitary females would show higher
114 levels of amicable behaviour towards males, as solitary females would be more ready
115 to mate while philopatric females would defend their family territories even against
116 strange males.
117

METHODS

Study species

The African striped mouse (*Rhabdomys pumilio*) is a communal breeder with non-breeding helpers at the nest (Schradin et all. 2012b). Groups consist of one breeding male (immigrated from another group), up to four closely related communally breeding females, and up to 25 adult young male and female philopatrics that act as helpers at the nest (Schradin et al. 2012b). Striped mice breed in spring (August/September to November/December) and most individuals born during the breeding season remain philopatric as young adults (>6 weeks old) in their natal group, where they stay for the entire dry season (December-April) and the cold wet winter (May-July). Some of the young adult philopatrics are able to successfully reproduce in the season of their birth by mating with partners from outside of the extended family group (males: Schradin & Lindholm, 2011; females: Schradin et al., 2010b), but the vast majority of young adults that remain as philopatrics do not reproduce until the next breeding season when they are one year old (Schradin et al., 2010b). At this age, males disperse and attempt to immigrate into groups of communally breeding females, while females remain philopatric in their group and breed communally. In both sexes, solitary breeding also occurs as an alternative tactic, and individuals can leave their group as young as 4-6 weeks of age when free territories are available (Schoepf & Schradin, 2012a). Less than 1% of striped mice survive for a second breeding season. Thus, most females breed only during one breeding season when they are 1 year old, and produce 2-3 litters during this breeding season.

Sexual Maturity

We conducted an experiment to confirm whether a perforate vagina was indicative of sexual maturity in our study species. At the University of the Witwatersrand, South Africa, we assessed the mating behaviour of 32 young females that had a perforate vagina for the first time by pairing them individually with sexually mature and experienced males. These females were housed in their family groups prior to pairing, and vaginal smears were examined daily from the day they were first perforate for a maximum of 4 days or unless they displayed oestrus. For the smears, we used the pipette lavage method which was minimally stressful and did not cause vaginal trauma in any of the females. The female was restrained with a glove and a small plastic pipette with a rounded tip containing a few drops of isotonic saline was inserted approximately 5 mm into the vagina. The fluid was expelled and immediately sucked up. The cell contents in the saline were transferred onto a clean microscopic glass slide. The procedure was repeated 3 times to ensure adequate numbers of cells. The procedure from restraining to release of the female was about 90 seconds. The slides were air dried and stained with Crystal violet stain. The cell composition of the smears was evaluated by light microscopy for the relative abundance of cornified epithelial cells, leucocytes and macrophages. Smears were obtained in the morning before 09h00 and females in oestrus (superabundant cornified epithelial cells; Byers et al., 2012) were placed in a neutral tank with a mature male for 30 min at 11h00, and the occurrence of lordosis (female), mounting and intromission were recorded.

Animals in Zurich

The colony consisted of animals originally trapped in 2002 in the Succulent Karoo (Goegap Nature Reserve) in South Africa. Animals were bred at the research station

in Goegap under natural weather conditions and F10 descendants were exported to the University of Zurich where a colony was established in October 2006.

Housing Conditions

Animals were kept at the University of Zurich under a 11.5:12.5h light regime and a temperature of approx. 22° C. Families were kept in two glass tanks 50x30x30cm which were connected to one another with a flexible plastic tube. A second tube connected to one type 4 plastic cage 20x13x15cm where a water bottle was provided (Fig. 1). Single individuals were kept in one glass tank connected to two type 4 plastic cages (Fig. 1). All tanks and cages had 5cm of wood shavings as bedding. The tanks additionally contained natural branches for environmental enrichment. Furthermore, each family and each singly housed mouse had one running wheel, which reduce stereotypic behaviour (pilot study).

Each family and each singly housed female had access to an extra enriched tank of 70x50x35cm for 1-2 days a week (Fig. 1). Connection was made by removing one type 4 cage and replacing it with another type 4 cage which was connected by flexible tube both to the home tank as well as to the enriched tank. The enriched tank was provided with bedding, tubes and natural branches. Up to five families and single female mice had access to one enriched tank on different days, such that the mice experienced olfactory cues of unrelated/unfamiliar striped mice.

Wild rodents kept in captivity are prone to develop stereotypic behaviour (for striped mice see Jones et al., 2010) which is known to affect both social behaviour as well as physiology and brain structure (Würbel, 2001). Thus, all striped mice were kept under super-enriched conditions which were successful in preventing the development of stereotypic behaviour (see below). Females of 15 sister pairs were

observed at the end of experiments (10 weeks old) for 15min in their home cage to determine whether mice had developed stereotypic behaviour. To determine whether the experimental setup was successful in avoiding stereotypic behaviours, we recorded whether mice showed flips, jumping in the corner or bar gnawing. Furthermore, the total time spent running in the wheel was measured. None of the 15 family housed females and none of the 15 singly housed females showed any stereotypic behaviours. Wheel running (measured in seconds/15min) was observed in only 5 family-housed and in 2 singly housed females and did not differ between the two female categories (59.8 $\pm$ 115.2 s versus 13.1 $\pm$ 45.4 s; T=4, W=13, Wilcoxon-test; p=0.22).

Mice were supplied with water *ad libitum*. Striped mice in the Succulent Karoo increase their body weight during spring and lose more than 10% weight during the following dry season (Schradin et al. 2012b). This might explain why they are prone to extreme obesity in captivity. To avoid obesity and as a form of enrichment, striped mice were not fed *ad libitum* but using the following schedule: a seed mix of 4.0g/individual (guinea pig and hamster food, Haefliger AG, Herzogenbuchsee, Switzerland) in the morning; one piece (approx. 1.0 g) of fruit or vegetable per individual at noon; and two mealworms per individual in the afternoon. Striped mice do not compete with each other for food, do not monopolise food (Schubert et al, 2009), and the growth rate was similar to captive mice fed ad libitum (Brooks, 1982) and faster than in mice in the field (Schradin, unpubl. data). This procedure was approved by the kantonale Veterinäramt in Switzerland as it reduces obesity and obesity related diseases such as diabetes (both are common in striped mice colonies fed ad libitum). The amount was known from many years of experience in South Africa.

Experimental Procedure

In total, 17 families took part in the study. Families were kept together until offspring were three weeks old (D21) and weaned (juveniles). At this stage, only one male and one female offspring remained with the pair. These were the individuals of the *family* treatment. They remained with their parents and also experienced raising the next litter. To avoid crowding in family cages, all juveniles except one male and one female of the second litter were removed when they reached 3 weeks of age. No pair had a third litter within the study period of 10 weeks.

Of the juveniles that were removed at 3 weeks of age, one female was housed alone, as described above. We therefore used a paired data design by randomly assigning one sister to being family housed and the other sister being singly housed. Some of the other individuals were used for a similar study in males (Schradin et al. 2013) or kept in sibling groups.

The female from the family and single treatment were weighed once a week and their reproductive state determined until both females had a perforate vagina. For this, individuals were removed by hand from their tank, inspected visually and returned within 2 minutes. Female rodents, including female striped mice, are regarded as being sexually mature and ready to mate when their vagina is perforate (open) (Brooks, 1982; this study), which we also use in field studies to determine sexual maturity and readiness to mate (Schoepf & Schradin, 2012a). When a singly housed female showed a perforate vagina before her sister did, a blood sample was taken from each to compare their corticosterone levels. Blood samples were taken the day after the difference was found to avoid a stress response due to handling during inspection. Samples were obtained within three minutes: the females were

anaesthetized using Methoxyfluran as suggested by the ethical committee in Switzerland (1ml on cotton-wool in a 300 ml chamber, with wire mesh between the animal and the anaesthetic). Methoxyfluran is very similar to Isofluran but always used without a vaporiser. A blood sample of 100-300 µl (depending on body mass) was taken from a tongue vein (sublingual blood sampling; Heimann et al. 2009), the preferred method of the Swiss Veterinary Council for taking blood samples from mice. We never had any indication that mice were negatively influenced by blood sampling. We were careful not to take too much blood, which is also the reason why we only had enough serum for one, not both hormone measurements for many individuals. The animals were returned to their home cage after 5min, when they had recovered from the slight anaesthesia. Blood samples stood at room temperature for 1.5 h and where then centrifuged for 10 min. The resulting serum was pipetted and frozen in aliquots.

Behavioural Testing

Behavioural data were collected from 15 sister pairs. Females were family or singly housed until they were 10 weeks old. We did two encounter tests per female on two different days, one with an unrelated female and another with an unrelated male. Both sisters were always tested on the same two days. For 8 of the sister pairs, a same-sex test was done on the first day and an opposite-sex encounter test was done on the second day, and the reverse was done for the other 7 sister pairs.

Social behaviours in rodents are scent dependent and direct interactions (grooming, sitting in body contact) cannot be observed when separating the mice with a wire mesh (Schradin & Pillay, 2004). From a previous study with young adult males we expected very little aggression (Schradin et al., 2010b) and this was supported by

267 our study (less than 50 % of mice showed any aggression). After tests, animals were
268 returned to their home cage and immediately checked, they were checked again a few
269 hours later (during noon when experiments were done in the morning; at 18:00 if
270 experiments were done in the afternoon), and no indication of long-lasting effects
271 (such as piloerection, sitting in one corner) were seen; instead mice were eating
272 normally.

273 Stimulus animals in these tests were all adults housed in sibling groups. For all
274 tests, we chose stimulus animals that weighed less than the test animal because
275 dominance is weight related in striped mice. Thus, the test animal was provided with
276 the opportunity to dominate the stimulus subject, and in this way we tested for the
277 motivation of the test and not the stimulus animal. For each sister pair, two different
278 stimulus animals from the same sibling group (same cage) were used and chosen
279 randomly. In half of the cases, the mouse from the family treatment was tested first,
280 while in the other half the singly housed sister was tested first.

281 All tests were performed in a neutral presentation arena 80x40x60cm made of
282 wood. At the beginning of tests, a partition in the middle divided the arena in two
283 compartments: we placed the stimulus mouse on one side of the partition and the test
284 mouse on the other. After an acclimation period of 5min, the partition was removed
285 and we scored the behaviour of the test mouse (focal animal sampling) for 15 min,
286 which was found in previous studies (Schradin et al., 2010b; 2013) to be long enough
287 to observe encounters also in shy individuals. We recorded the frequency of
288 aggressive behaviours (fight, bite, chasing) as well as grooming of the stimulus mouse
289 by the test mouse. We used the same experimental procedure in previous studies
290 (Schradin et al, 2010b; Schradin et al., 2013) and experiments would have been
291 terminated when damaging fights would have occurred where one mouse tried to bite

another one (termination criteria). However, in the present study, biting was never observed. The total time spent in body contact with each other was also measured. For nine sister pairs, a blood sample was collected and processed as described above for hormone measurements two days after the last encounter.

Hormone Assays

Blood samples were analysed in the EIA laboratory of the Zoological Institute, University of Zurich. Commercial kits from IBL Hamburg were used. Procedures were as stated in the kit manuals. However, due to high corticosterone levels typical of this species, samples were diluted 1:99. All measurements were well within the standard curve of the assay. Serial dilution of striped mouse saple pools paralleled the standard curve and the slopes were not different. Intra- and inter-assay variability was determined with pools from striped mice that had low and medium high values. Eight measurements were done for intra-assay and five for inter-assay variability. Intra-assay variability was 8.3 and 22.3 % for the medium and low pool respectively. Inter-assay variability was 6.4 and 2.3 %. Progesterone values were measured in one single assay. Intra-assay variability for two pools (8 and 10 samples) was 4. 4% and 5.0 %. We did not have sufficient amounts to measure both corticosterone and progesterone for all samples, and in these cases we focused on corticosterone, resulting in lower sample sizes for progesterone.

Ethical Note

The research adhered to the ASAB/ABS Guidelines for the Use of Animals in Research. We provided animals with environmental enrichment (as described above). The welfare of the animals was monitored by checked them three times a day visually

317 during feeding, and by behavioural observations demonstrating the absence of
318 stereotypic behaviours. The experimental procedures used did not have any obvious
319 negative effects on the welfare of the striped mice. Singly kept females represented
320 the solitary kept tactic observed in nature, and by providing females access to a tank
321 which on other days was also used by different striped mice, females were not isolated
322 olfactorily from conspecifics. Thus, our highly enriched conditions mimicked the
323 situation of solitary females in the field. Thus, at the end of the experiment, all
324 experimental animals were euthanized (family and singly-housed females) by
325 anaesthetizing them with methoxyflurane and euthanizing them by cervical
326 dislocation followed by decapitation. The rest of the family group remained in the
327 breeding stock of the colony. Animal ethical clearance was provided by the Kantonale
328 Veterinärmt of the Kanton Zürich in Switzerland (ethical clearance number 91/2006)
329 and the University of the Witwatersrand (AESC number: 2012/13/2B).
330
331 *Statistical Analyses*
332 Data were analysed using Instat 3.05. A paired data design was followed using paired
333 t-tests. Behavioural data were often not normally distributed and thus analysed using
334 the non-parametric Wilcoxon matched-pairs rank sign test to test between sister pairs.
335 All tests were two tailed. Data are presented as mean $\pm$ SD.
336
337 **RESULTS**
338 *Perforate vagina, sexual maturity and readiness to mate in young philopatric females:*
339 Of the 32 females tested for mating behaviour when their vagina was perforate, 4
340 females were in oestrus on the day they were first perforate (day 0), 10 on day 1, 15
341 on day 2 and 3 on day 3. A total of 25 females displayed lordosis (78 %) and 20 of

these mated (63 % of all females, 80 % of those showing lordosis). The day of first oestrus (day 0-3) did not affect whether or not lordosis (χ^2_3 = 2.55, P = 0.47) or mating (χ^2_3 = 0.36, P = 0.95) occurred. Females that mated did not differ from those that did not mate in their age (30.25±4.96 days versus 31.42±3.61days; t_{30}=0.10, P =0.92) and body mass (37.01±5.47 g versus 39.42±4.78 g; t_{30}=0.31, P =0.76). Births were recorded in 13 females.

Comparison of Attainment of Sexual Maturity

Singly housed females did not differ from family housed females in the age at which they first were observed to have a perforate vagina (5.8 ± 1.5 weeks versus. 6.2 ± 1.7 weeks; N=17 sister pairs, paired t_{16}=0.79, P=0.44) nor in the body mass at which they first were observed to have a perforate vagina (31.0 ± 6.8 g versus 29.7 ± 5.5 g; N=17 sister pairs, paired t_{16}=0.81, P=0.43).

Comparison of Hormone Levels

To test the hypothesis that high corticosterone levels delay the onset of female reproductive maturity, we compared corticosterone levels between family housed females that showed a perforate vagina later than their singly housed sisters. In doing so, we increased the power to detect the predicted difference, by excluding sister pairs with no differences in timing of perforate vagina. The same experimental design was used to test whether hormonal differences explain the timing of sexual maturity in brothers housed in a group and alone (Schradin et al 2009). Sister pairs, like the brother pairs previously, that did not differ in the timing of maturity were excluded for ethical reasons to avoid blood sampling when no difference was expected. In contrast to our expectation, we found no difference in age of sexual maturity (see above),

367 which we did not know before we determined our sample criteria. We analysed the
368 hormones of those sisters from which we collected blood samples to separate the
369 effects of hormonal influence and statistical noise.

370 Singly housed females that had a perforate vagina before their family housed
371 sisters did not differ in corticosterone levels from their sisters at this stage (age of on
372 average 6 weeks; 1039 $\pm$ 610 ng/ml versus 912 $\pm$ 596 ng/ml; N=6 sister pairs, paired
373 t_5=0.56, P=0.60). Singly housed females did not differ from family housed females in
374 corticosterone levels at 10 weeks of age (732 $\pm$ 401 ng/ml versus 781 $\pm$ 331 ng/ml;
375 N=10 sister pairs, paired t_9=0.34, P=0.74) when experiments were terminated.

376 For progesterone, only samples from 8 females of 4 of the 6 sister pairs were
377 available for the period when females differed in timing of showing a perforate
378 vagina. All females had measurable progesterone levels (singly house: 7.0 $\pm$ 4.6
379 ng/ml; family housed: 2.2 $\pm$ 1.2 ng/ml), but no statistical comparisons were possible.
380 Singly housed females did not differ from family housed females in progesterone
381 levels at 10 weeks of age (4.8 $\pm$ 3.0 ng/ml versus 9.9 $\pm$ 20.6 ng/ml; N=8 sister pairs,
382 paired t_7=0.73, P=0.49) at the end of experiments.

383

384 *Social Interactions with other Females at 10 Weeks of Age*

385 Singly housed females groomed the stimulus females significantly more often than
386 did family housed females (5.7 $\pm$ 5.4 times/300 s versus 0.1 $\pm$ 0.4 times /300 s; N=15
387 sister pairs, T=0, W=-120, Wilcoxon-test, P<0.0001; Fig. 2a), they spent significantly
388 more time in body contact with them (314.7 $\pm$ 186.4s /300 s versus 55.0 $\pm$ 148.091s
389 /300 s; N=15 sister pairs, T=6.5, W=-107, Wilcoxon-test, P=0.001; Fig. 2b), and they
390 showed significantly less aggression towards them (0.3 $\pm$ 0.7 times/300 s versus 2.0 $\pm$
391 2.6 times /300 s; N=15 sister pairs, T=0, W=45, Wilcoxon-test, P=0.004 Fig. 2c).

Aggression was rare: in 6 sister pairs, none of the females showed aggression, and only two of the singly housed and 9 of the family housed females showed some aggression (chasing; Fig. 2).

Social Interactions with Males at 10 Weeks of Age

Singly housed females groomed the stimulus males significantly more often than did family housed females (7.3 $\pm$ 7.4 times/300 s versus 0.6 $\pm$ 1.1 times/300 s; N=15 sister pairs, T=1, W=-76, Wilcoxon-test, P=0.001; Fig. 2d), they spent significantly more time in body contact with them (280.3 $\pm$ 220.4 s /300 s versus 135.1 $\pm$ 173.3 s /300 s; N=15 sister pairs, T=17, W=-71, Wilcoxon-test, P=0.02; Fig. 2e), and they showed significantly less aggression towards them (0.5 $\pm$ 0.8 times/300 s versus 2.3 $\pm$ 2.8 times /300 s; N=15 sister pairs, T=-5, W=45, Wilcoxon-test, P=0.02; Fig. 2f). No mating or attempts of mating were observed.

Response towards stimulus males versus stimulus females: Family housed females did not groom the stimulus males significantly more often than the stimulus females (N=15 sister pairs, T=6, W=-16, Wilcoxon-test, p=0.22), nor did they differ in their aggression levels towards the two sexes (N=15sister pairs, T=-32.5, W=1, Wilcoxon-test, P=0.97). However, they spent significantly more time in body contact with males than with females (N=15 sister pairs, T=18, W=-69, Wilcoxon-test, P=0.03).

Singly housed females did not groom the stimulus males more often than the stimulus females (N=15 sister pairs, T=31, W=-43, Wilcoxon-test, P=0.19), they did not spend significantly more time in body contact with them (N=15 sister pairs, T=-38, W=44, Wilcoxon-test, P=0.23), nor did they show less aggression towards them (N=15 sister pairs, T=13, W=-10, Wilcoxon-test, P=0.55).

DISCUSSION

Physiological reproductive suppression of subordinate females occurs in many cooperatively and communally breeding mammals (Abbott, 1984; Brant et al., 1998; Clark & Galef, 2001; Getz et al., 1983; Moehlman & Hofer, 1997; Saltzman et al., 2006; Savage et al., 1988; Solomon et al., 2001), but we found no evidence for this in the communally breeding striped mouse. Thus, there must be other proximate and ultimate reasons for why young females often do not breed, which could include stress induced by high population density, inbreeding avoidance, and energy allocation trade-offs between growth / survival and reproduction. Behaviourally, we found significant differences between philopatric and solitary female striped mice, with singly housed females being more amicable and less aggressive to strangers than their family housed sisters.

Both family housed females and their singly housed sisters had a perforate vagina at approximately 6 weeks of age. In an additional experiment, we were able to demonstrate that newly perforate females were sexually mature, showed lordosis and were ready to mate, supporting the assumption of previous studies that a perforate vagina is an indication of sexual maturity (Perrin & Johnson, 1999; Willan, 1982). Importantly, the females in our study were young adult philopatrics living in their family group which readily mated when in oestrus, even though in their family they would not reproduce due to inbreeding avoidance (Pillay, 2002). Interestingly, in the field, young females reproduce when population density is low, and they do so with males outside their family group (Schradin et al., 2010b).

In accordance with our morphological and behavioural data, our progesterone data indicate that females were physiologically ready to reproduce, as all females had

clearly measurable progesterone levels. This was also represented in the vaginal smears, indicating oestrus in young females living in their natal family group. For the closely related sister species *R. dilectus* (previously regarded as the same species *R. pumilio*; Rambau & Robinson, 2003), females have been reported to reach sexual maturity at an earliest age of 5 weeks in the field (Pillay, 2002) and 6 weeks in captivity (Brooks, 1982), which fits the results of our present study where females were 5-6 weeks old when the vagina was first perforate. We found no differences in corticosterone or progesterone levels between philopatric and singly housed females, indicating that both classes differed neither morphologically nor physiologically in the age when they reached sexual maturity.

In conclusion, there was no evidence for reproductive suppression of philopatric females. Using the same experimental approach, multiple studies have shown that male philopatric striped mice are sexually suppressed (Schradin et al., 2009; Schradin et al., 2012a; Schradin et al., 2013). Thus, we do not believe that the experimental set-up can explain why no reproductive suppression was observed in females. Sex-differences in the absence / presence of reproductive suppression indicate a more egalitarian social structure for females than for males, which is reflected in the field by each group having only one breeding male but up to four breeding females (Schradin et al. 2012b). Support for this egalitarian relationship between females was provided in another study which did not show competition for food between sisters (Schubert et al., 2009).

In the field, the percentage of young adult females that breed in the season of their birth varies from year to year, probably as a function of population density. In years with very high population density, almost no young adult female reproduces (Schradin et al. 2012b), in years with intermediate population density 28% of young

467 adult females reproduce, and in years with low population density 90% of young adult
468 females breed (Schradin et al., 2010b). Our study cannot rule out the possibility of
469 reproductive suppression under high population densities. Mice were kept in family
470 groups with only one breeding female, which was the mother of the philopatric
471 female, representing low population density in the field. Under high population
472 density, 2-4 older females breed communally in a group. It is possible that the
473 presence of several breeding females, especially aunts, which are less closely related
474 to young philopatric females than their mothers, induces sexual suppression.
475 Communal groups are characterized by competition between the breeding females
476 (Schradin et al., 2010a), and it is possible that the older breeders will reproductively
477 suppress young females.

478 High population density will likely increase the frequency of inter-group
479 territorial encounters, which are typically lost by smaller individuals such as young
480 adult females. Thus, the absence of reproduction in young adult females could be due
481 to territorial aggression inducing stress, or increased within group competition, as the
482 number of breeding females per group correlates with population density (Schradin et
483 al., 2010a). Another reason could be behavioural suppression and female-female
484 aggression within communal groups, which has been reported repeatedly (Schradin et
485 al., 2010a; Schubert et al., 2009). A third reason for free-living philopatric females not
486 breeding might be inbreeding avoidance. In captivity, females do not breed with a
487 familiar breeding male of their group regardless of genetic relatedness (Pillay, 2002)
488 and in the field, young adult females avoid mating with the breeding male of their
489 group and seek copulations with males outside of their natal group (Schradin et al.,
490 2010b). A fourth possibility is a trade-off in energy allocation. It might be more
491 beneficial for young females to invest in growth and survival than in reproduction.

492 Thus, a combination of aggression experienced both by females from neighbouring
493 groups and older females within the group together with inbreeding avoidance and
494 resource allocation might explain why many young adult striped mouse females do
495 not breed in their natal group, but these factors need future examination.

496 In our study, singly housed females were more amicable and less aggressive
497 towards strangers of both sexes than their family housed sisters. In contrast, in the
498 field, aggressive philopatric females were more likely to become solitary, and solitary
499 striped mice (both sexes combined) were more aggressive towards same sex
500 individuals (Schoepf & Schradin, 2012b). Most (75%) of the females that became
501 solitary in the field did also reproduce but none of our singly housed females had an
502 opportunity to breed. Aggressive behaviour of solitary females in the field might have
503 been related to hormonal changes associated with pregnancy, which is known to
504 increase aggression (Rosenblatt et al., 1994).

505 Singly housed females were more amicable and less aggressive towards males
506 than were the philopatric females, and the same levels of tolerance was found for
507 strange females. Ontogenic changes in social behaviour can occur pre- and post-
508 dispersal. In solitary species, affiliative behaviour pre-dispersal can be followed by
509 intolerance of and aggression directed at conspecifics around dispersal and thereafter
510 (Schoepf & Schradin, 2012b). Affilliative behaviour in group-living species is
511 typically restricted to group members (Smith et al., 2012), and aggression can occur at
512 maturity because of competition for positions on a dominance hierarchy (Walters,
513 1980). Whereas xenophobia is common in group-living species, tolerance of strangers
514 as an adaptive response to form new groups can occur (Moore, 1984; Ganem &
515 Bennett, 2004). The high levels of amicable and low levels of aggressive behaviours
516 in solitary kept females might indicate a general tendency to sociality, which could

517 explain why striped mice always choose to live in groups in periods without
518 reproductive competition, as occurs during the non-breeding season (Schoepf &
519 Schradin, 2012a; Schradin et al., 2010a).

520 Our study indicates that living as a young adult in a family group has very
521 different consequences for male and female striped mice. While several previous
522 studies demonstrated reproductive suppression in philopatric males living in family
523 groups, this does not occur in female striped mice. The alternative explanations for
524 why many young adult females do not breed, even though they might be
525 physiologically capable to do so, are territorial aggression, within group competition
526 between breeding females, inbreeding avoidance, and energy allocation trade-offs
527 between reproduction and growth / survival. Future studies will have to test these
528 factors. Still, reproductive suppression might occur under high population density in
529 communally breeding groups. Additionally, our study indicates that being housed
530 alone induces behavioural changes in young adult females, which become more
531 amicable and less aggressive.

532 In conclusion, our study demonstrates the high flexibility of social behaviour
533 in striped mice which can change their reproductive and social tactics depending on
534 variable ecological conditions (Schradin et al., 2012b). The reason that females
535 choose to disperse and breed solitarily instead of breeding communally is apparently
536 not to avoid physiological reproductive suppression in family groups, as young
537 females are not reproductively suppressed, though we cannot exclude the possibility
538 that this occurs under high population density. Staying in the natal group as a young
539 non-reproducing female can be regarded as a real alternative reproductive tactic,
540 which avoids costs of reproductive competition within groups in the form of
541 infanticide and female-female aggression (Schradin et al., 2010a). This tactic is

predicted to lead to increased survival due to benefits of group-living and ultimately increased lifetime reproductive success.

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683 Figure legends

684

685 **Figure 1**

686 Experimental setup. Left: a family setup consisting of two glass tanks and a cage
687 connected by flexible tubes. Right: a single individual setup consisting of one glass
688 tank and one cage. Running wheels were provided for each group / single individual,
689 which were also allowed ones a week to enter an enriched tank (top left). This highly
690 enriched setup was successful in preventing stereotypic behaviour.

691

692 **Figure 2.** Behavioural differences between family housed females and their singly
693 housed sisters. The data of each sister pair are connected by a line. a, b and c:
694 behaviours shown towards an unfamiliar female in a neutral presentation arena. d, e
695 and f: the same behaviours shown towards strange males in a neutral presentation
696 arena. Top row (a and d): grooming. Middle row (b and e): sitting in body contact.
697 Bottom row (c and f): aggression. All comparisons were significant ($p \leq 0.02$).

698

699

700 

701 **Figure 1 (black and white for print version, please!)**

702

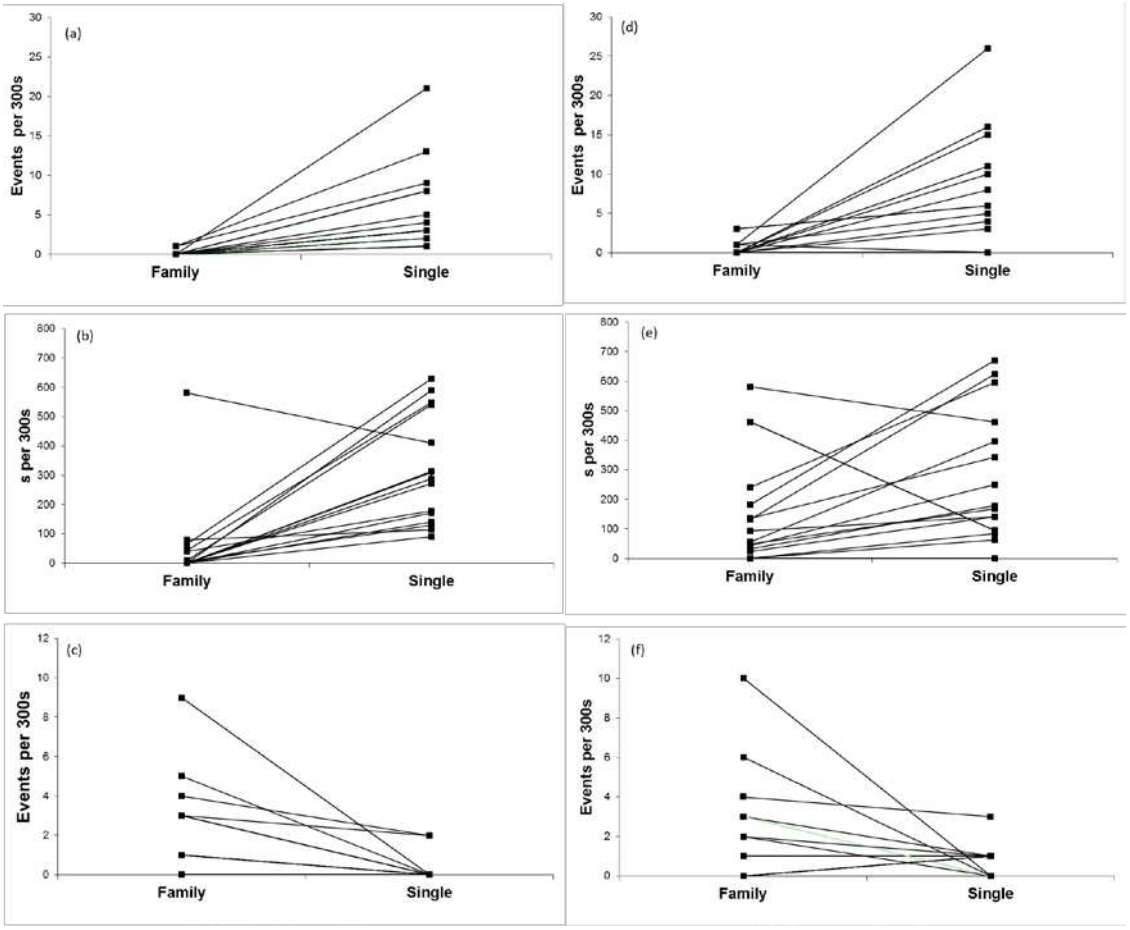
(a)
Events per 300s
Family
Single
(d)
Events per 300s
Family
Single
(b)
s per 300s
Family
Single
(e)
s per 300s
Family
Single
(c)
Events per 300s
Family
Single
(f)
Events per 300s
Family
Single

Fig. 2